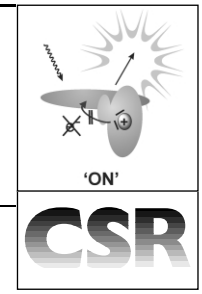
Rigidization, preorientation and electronic decoupling—the 'magic triangle' for the design of highly efficient fluorescent sensors and switches



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One of the key interests in the recent development of fluorescent molecular sensors and switches is the realization of systems that show strong signal changes as a response to the chemical trigger. Aiming at rational probe design, this article compiles and compares different promising strategies to extract those supramolecular and photophysical features that allow the construction of molecular devices suitable for efficient signaling. The examples comprise fluorescence 'OFF'–'ON' as well as 'ON'–'OFF' operative systems and the mechanisms, properties, and limitations of the different design concepts are discussed.

1 Introduction

In biology, chemistry, and material sciences, analyte recognition, the determination of local environmental properties or the operation of molecular-scale switches require the efficient transduction of an event into a measurable signal. Depending on the actual location of the source and the properties of the surrounding medium, the signal generated by such an event should ideally be detectable both in close vicinity as well as at remote distances and should strongly differ from any signals from an unspecific background, the unbound sensor, or the switch in the 'ZERO' or 'OFF' position. Moreover, a rational application implies that the desired information is only reported upon request. Random and uncontrolled generation of the analytically important output by, for instance, changes of other environmental physical parameters or by unintended chemical processes, which are likely to occur in the biological, natural or artificial medium of operation, have to be avoided. Besides a certain specificity towards the target, this prerequisite requires an immunity of the reporter unit against stimulation by its immediate periphery as well as auto-stimulation.

A versatile means of communication to access information in such a way is light, in particular, luminescence. Luminescence spectroscopy operates on the basis of 'call-and-response': the requested information, a fluorescence signal of a certain intensity, spectral and temporal distribution, is only obtained after excitation of the potentially fluorescent label or molecule, usually within the spectral region of its energetically lowest-lying allowed transition between ground and first excited singlet state. The intrinsic selectivity of communicating *via* two experimental parameters, excitation and emission wavelength, along with the high sensitivity (down to the single molecule-level) and the possibility of increasing the recognition ability by monitoring fluorescence additionally in the temporal regime or by accessing a signal *via* remote control with waveguides and

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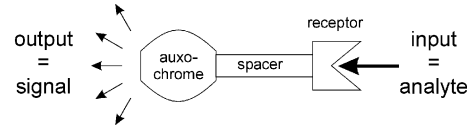
fiber optics have led to the wide-spread use of fluorescence techniques in chemistry-related sensing and switching applications.[1]

Focusing on the design of highly efficient fluorescent sensors and switches in this review, we will leave aside topics such as sensing of natively fluorescent analytes or bio- and chemiluminescence methods. Moreover, as a central aim of the present article is to discuss structural aspects of reporter design within the framework of supramolecular chemistry and dye photophysics, we will concentrate on actual chemical species as photo-triggers and will not go into detail on fluorescent probes for the micro-environment.[2] For parameters such as solvent polarity or medium viscosity, the analytical or operational task is opposite to that in most other molecular indicators and thus, the construction of efficient probes for these purposes has to follow different logics. Whereas powerful switches should allow discrimination between two states, 'ON' and 'OFF', the ideal probe for the local environment should efficiently indicate gradual changes. Furthermore, the latter task predominantly requires optimization of the fluorophore and can dispense with constructing or tuning specific receptor units or properties.

However, the fluorometric indication of non-fluorescent substrates is still too large a research area to be comprehensively covered here.[3–5] Thus, in this contribution, we focus on molecular fluorescent sensors and switches that respond to small inorganic cations (metal ions, protons) and the reader is referred to refs. 6 and 7 for recent developments in fluorescence-based signaling of other analytes (*e.g.*, anions[6] or neutral organic molecules[7]) as well as to ref. 8 for the introduction of new design concepts such as sensory molecular polymeric materials.

2 Key concepts and commonly employed design principles

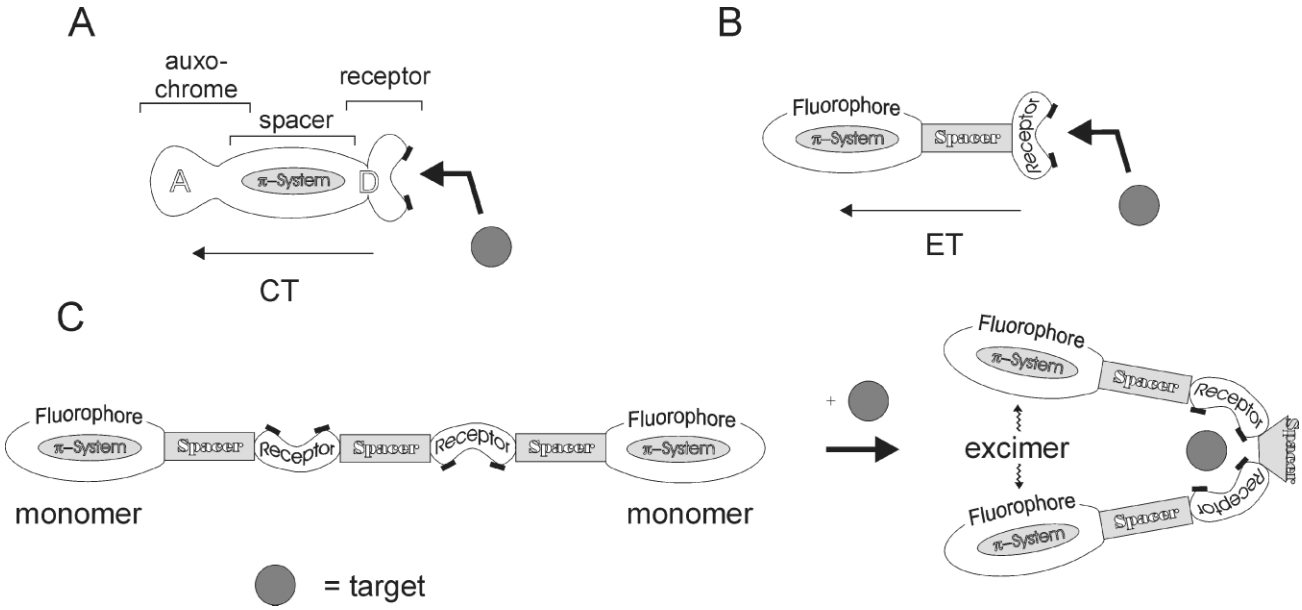
Besides supramolecular and molecular recognition chemistry, which are at the heart of tailor-making or optimizing the chemical selectivity and sensitivity of fluorescent molecular sensing devices, the tuning of the signaling features is predominantly connected to dye architecture and photophysics. To be able to control and tune both properties as independently as possible, a composite constitution of the reporter molecule is chosen in most cases. As is schematically depicted in Scheme 1,



Scheme 1 Operating principle of analyte-responsive auxochrome–spacer–receptor systems.

the common molecular fluorosensor consists of an auxochrome as the key component for generating the fluorescence signal and an analyte-responsive receptor, both of which are linked by a spacer.[3,4,9] Depending on the underlying photophysical mechanism, the spacer can be saturated to deconnect the electron systems of chromophore and binding unit or unsaturated to couple these electronic subsystems. Most composite fluorescent molecular reporters for protons and metal ions principally belong to one of the following three groups (Scheme 2): *intrinsic*, (photoinduced) *electron transfer*- (ET-) operative, or *exciplex* or *excimer* forming fluorescent probes.[3,9] As this review does not contain examples of sensor molecules that operate through other signaling mechanisms, as for instance various types of energy transfer, such mechanisms will not be detailed here and the reader is referred to refs. 3,4,9 and other recent reviews for further examples.

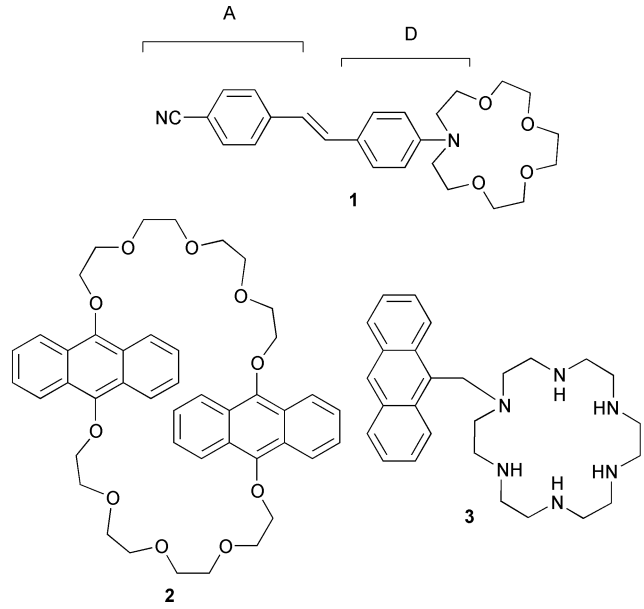
For intrinsic probes, ligating site and main chromophore are in direct electronic conjugation. The subunits are chosen in such a way that they act as donor and acceptor modules of a system thus capable of undergoing intramolecular charge-transfer (CT) transitions, at least in the usually highly polar media of application. The recognition event, which takes place at the donor or the acceptor moiety and accordingly influences donor or acceptor strength, is transduced by an analyte-mediated change of the CT process. In the case of ET probes, the binding and the signaling unit are usually separated by a (short) alkyl spacer, electronically disconnecting the π electron systems of receptor and fluorophore. In the unbound state, after excitation, a fast electron transfer from or to the fluorophore quenches the fluorescence of the system. Analyte binding at the receptor modulates its redox potential and slows down (or completely 'switches off') the ET process, reviving fluorescence emission. Besides substrate-enhanced fluorescence, this indication logic can also be reversed. In this case, the 'switched on' free sensor molecule forms a weakly emissive probe–analyte associate. The third class of fluorescent reporters is based on flexible dye architectures that enable the formation of an intramolecular



Scheme 2 Schematic representation of the most common types of the three main classes of fluorescent molecular reporters for small inorganic cations: **A**—intrinsic and **B**—ET-operative systems with an electron-rich receptor; **C**—excimer forming probe with receptor units integrated in the spacer.

exciplex or excimer between fluorophore and/or receptor units. Besides flexibility, the constitution of this supramolecular system should allow strong intramolecular geometry changes upon complexation of the analyte, thus modifying the ratio of excimer/exciplex-to-monomer emission. Since the various details of the underlying mechanisms have been extensively reviewed recently,[3–5,9] we will only elaborate on those details which are essential for understanding the motivation of and the improvements gained by designing fluorescent sensors and switches according to the presented strategies.

From a chemical point of view, up to now, the most prominent classes of chromophores used in sensor molecules are polyaromatic hydrocarbons (*e.g.*, anthracene, pyrene) and heterocyclic systems (*e.g.*, coumarins, cyanines, fluoresceins). On the other hand, receptor sites are mainly recruited from heteromacrocyclic chemistry (*e.g.*, open chain polyethers or polyamines, crown ethers, cryptands) or from the group of 'classical' chelating agents (*e.g.*, glycol-bis(β-aminoethyl ether) tetracetic acid, EGTA, or 1,2-bis(2-aminophenoxy)-ethane tetracetic acid, BAPTA, analogues).



3 Requirements for efficient signal transduction

In terms of highly efficient signal generation and transduction, the indication of a binding event is best achieved upon (selective) conversion of a (very) weakly fluorescent molecule (the 'OFF' state) into a brightly fluorescent species, the 'ON' state. The measurement of a strong or enhanced fluorescence is analytically favored as compared to a (analyte-induced) decrease in emission intensity, since the former case displays a higher signal-to-noise ratio and is commonly accompanied by the appearance of a characteristic new and longer-lived fluorescence decay component. This increase in lifetime can, for instance, be used to suppress background fluorescence or to discriminate between analyte–probe complexes with different distinct decay times.[9] (Very) strongly emitting species additionally offer the possibility to employ fluorescence detection in the single molecule domain.[1] However, many of the molecular fluorescent sensors and switches which were designed according to the principles introduced in Section 2 show only rather weak fluorescence changes and often even fluorescence quenching as a result of the complexation or protonation reaction.[3,4,9]

To obtain a better understanding of the structural and electronic requirements for powerful molecular probes, let us inspect the three types of reporters introduced above in the light of key components and mechanistic prerequisites. For intrinsic probes, where all the subunits form the chromo/fluorophore, many commonly used receptor units with a nitrogen as the bridge head atom (*e.g.*, mono- or polyaza macrocycles) are sufficiently electron-rich to trigger fast CT processes. However, since the general aim is to construct fluorescent probes that absorb and emit in an analytically advantageous wavelength region, *i.e.*, the visible–near infrared (NIR), extensive π-systems of at least stilbene, styryl or cyanine type are required. The usual synthetic approaches to obtain such substituted intrinsic sensor systems yield chromophores with at least one unbridged, *i.e.*, non-fixed, double and/or single bond, while the electron donor (D) and acceptor (A) occupy the terminal positions of the conjugated π-system (see model compound **1**).[4] A high emissivity, essential for a bright signal, is thus critically determined by the intrinsic photophysical behavior of not fully rigidized D–A-substituted dyes with a central vinylene spacer. Unfortunately, based on the dynamics of excited charge-transfer states, most stilbene or styryl dyes with only a single unbridged bond in the spacer fragment are not highly fluorescent.[10] In addition, these intrinsic properties and excited-state reaction–deactivation mechanisms usually hamper strong proton- or cation-induced fluorescence changes, disqualifying most of these dyes as efficient fluorescent switches. (This disadvantage is found regardless of the position of the receptor, *i.e.*, whether it is integrated as D or A; a more detailed mechanistic description is given in ref. 9.) Thus, the concept of intrinsically connecting fluorophore and receptor seems not to be very suitable for our purpose. A similar situation is encountered with many exciplex/excimer forming sensor molecules. Although equipping them with both a powerful fluorophore and receptor is readily feasible, the spacer is the critical component. In the representative example **2**, the structure of the spacer, *i.e.* its length as well as the nature and position of its coordinating heteroatoms, is essential for bringing both parts of the exciplex/excimer pair into the required close contact upon complexation. Consequently, only very few guests are able to allow the complex to adopt the specific conformation necessary for efficient signal generation.[4]

Based on the characteristic feature of electron transfer processes which principally operate over relatively long distances,[11] in general, the class of ET-active fluorescent molecular sensors and switches seems to be best suited for the current task. This design also offers tremendous flexibility with respect to composing modular dye architectures. Upon the choice of adequate fluorophores and receptors, fast ET processes can easily be invoked and the analyte-induced switching factors obtained with comparatively simple compounds can already be very large (as an example, see **3**).[3] Unfortunately, for certain analytes, a simple constitution such as in **3** does not work. In the presence of heavy and transition metal ions such as Hg^{II}, Cu^{II} or Fe^{III} commonly acting as efficient fluorescence quenchers,[4,9] no enhanced emission signals are found for this molecule and the majority of related probes.[9] Apparently, in compounds such as **3**, the spacer and receptor units are too flexible to prevent an interaction between the potentially quenching guest and the host's fluorophore.

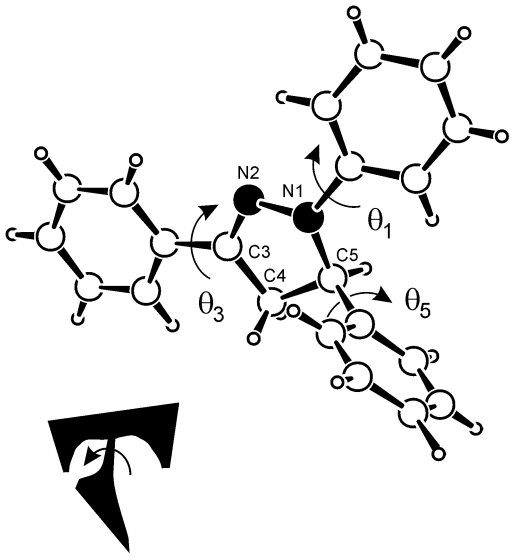
In summary, we can conclude that the key factors for efficient signal generation and transduction are (i) rigidization of all the critical parts of the molecular device and (ii) strict control of their preorientation. Closely connected to these structural features is a third requirement promoting switching efficiency, electronic decoupling of the functional subunits, the last corner stone of the 'magic triangle' addressed in the title of this article.

Before presenting some guidelines to improve molecular fluoro-switching operations, it is important to consider which tools are available to the chemist for rational probe design. Since the key features of many of the approaches reported

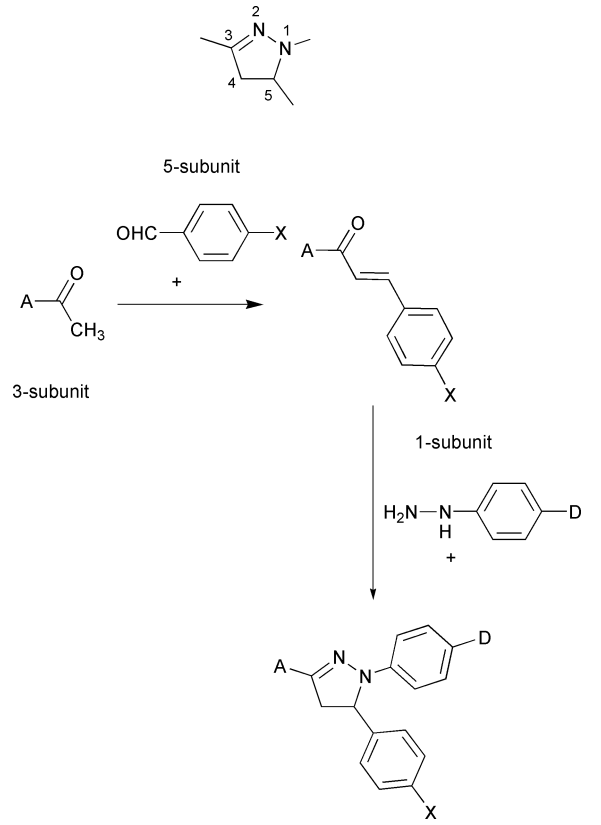
below are modularity and (the highest possible degree of) independence of the CT- or ET-active subunits, redox potentials are of major importance to the designer. Besides tabular compilations of electrochemical properties in combination with the relevant thermodynamic mathematical formalisms,[12] quantum chemical calculations can be helpful in desktop design (see W. Rettig *et al.* in ref. 1). On the experimental side, photophysical,[13] electrochemical,[14] NMR[15] or X-ray[16] investigations of model systems are very valuable to gain further insight into the processes and structures that distinguish advanced supramolecular functional dyes.

4 Rigidization and preorientation of the molecular subunits

Following the description given in the previous section, the first example of powerful fluorescent reporters reviewed here, 1,3,5-triaryl-Δ^2-pyrazolines, belongs to the class of ET-operative probes. The core of the architectural logic employed is a short but highly rigid spacer that keeps the active components fixed in a kind of 'twisted-T' conformation (Scheme 3). As the synthesis of these 1,3,5-triaryl-Δ^2-pyrazolines is basically a straightforward and stepwise combination of the single subunits (Scheme 4), this type of supramolecule provides a very versatile basis for the tailor-making of modular fluorescent indicators with different selectivities as well as optical properties, *i.e.*, for various wavelength regions.[12,14,17–19] The latter feature especially is a big advantage for these systems over conventional ET probes containing polyaromatic hydrocarbons as fluorophores. It is, for instance, possible to span the 400–800 nm emission range simply by variation of the substituent in the 3-position.[12,19] The tuning of the spectral properties is achieved by relying on a donor–acceptor-substituted charge-transfer fluorophore. In general, the sensor parameters of such compounds are largely predictable from the optical, electrochemical, and acid–base or cation complexing properties of the individual molecular modules. Moreover, a high fluorescence quantum yield in the 'ON' state is guaranteed by holding the CT chromophore not only in an arbitrarily fixed but also in a rigid and largely planar conformation. The detailed photophysical processes can be rationalized as follows.

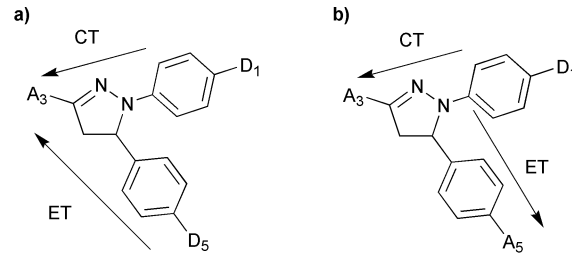


Scheme 3 Quasi 3-dimensional view of the 'twisted-T' conformation of 1,3,5-triaryl-Δ^2-pyrazolines based on X-ray structures reported in ref. 14. The twist angles indicate the preorientation of the subunits and are in the range of $\theta_1 \sim 15°$, $\theta_3 \sim 5°$, and $\theta_5 \sim 80°$.



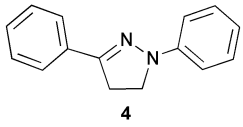
Scheme 4 General structural pattern and synthetic route to modular 1,3,5-triaryl-Δ^2-pyrazolines.

The rigid and highly fluorescent CT fluorophore is represented by the basic 1,3-diaryl-Δ^2-pyrazoline moiety, involving the ring fragment C(3)=N(2)–N(1) and the 1,3-substituents. The actual acceptor moiety comprises the fragment A_3–C(3)=N(2) and the donor consists of N(1)–D_1. The analytical signal is triggered by the spacer-appended receptor in the 5-position, which can be either an electron donor or acceptor. In contrast to the CT within the main chromophore, the non-conjugated fluorophore and binding modules can interact *via* an electron transfer (Scheme 5). Accordingly, all the spectroscopic and analytical properties of 1,3,5-triaryl-Δ^2-pyrazolines are governed and determined by the competition of fluorophore-based charge transfer and analyte-arrestable or -releasable receptor-to-fluorophore or fluorophore-to-receptor excited-state electron transfer.[12,14] The conformational separation of both ET-active sites is realized by the central 5-membered ring. Its unique electronic configuration, *i.e.*, the partly saturated character with sp^3-hybridized C(4) and C(5), holds the 5-receptor in a pseudo-spiro conformation, thus preorienting and electronically decoupling CT module and ET trigger.[14] The importance of careful supramolecular design is evident from a comparison of the



Scheme 5 Schematic constitution of A_3–D_1–D_5 and A_3–D_1–A_5 substituted 1,3,5-triaryl-Δ^2-pyrazolines and the respective directions of competing CT and ET processes.

model compounds **4**–**6**. Whereas **4** fluoresces with a quantum yield $\Phi_f = 0.92$ in benzene, **5** and **6** are both non-fluorescent in this solvent.[20] Only the 5-membered ring is rigid enough to prevent fast radiationless deactivation by intramolecular ring inversion as occurs in **5** or photochemical *syn–anti* isomerism as takes place in **6**. Furthermore, since the spacer of 1,3,5-triaryl-Δ^2-pyrazolines is chosen as short as possible, the excited state ET is efficiently mediated, enabling strong changes of the fluorescence output.



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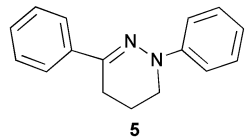

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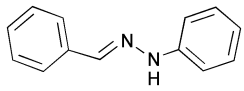

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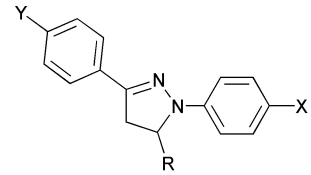
Depending on the intended direction of the substrate-responsive ET process, sensory 1,3,5-triaryl-Δ^2-pyrazolines for protons and metal ions can be realized by integrating either electron accepting or electron donating 5-substituents whose redox potentials are strongly affected by the binding event. The former design employing for instance pH sensitive aromatic carboxylates or pyridines relies on an excited-state ET from the pyrazoline moiety to the (protonated) receptor ('inverse' or 'ON'–'OFF' switching). The latter more conventional approach, using cation responsive anilino receptors, invokes a photoinduced ET from the unbound receptor to the pyrazoline chromophore ('normal' or 'OFF'–'ON' switching).

Two families of pH sensors of the general structure **7** have been realized with benzoic acid or pyridyl groups as 5-substituents, the most efficient one being **7a** with a de-protonation-induced fluorescence enhancement factor (FEF) of 108 in water–methanol.[12] Here, for a given 1,3-substitution pattern, the fluorescence quenching in the 'OFF' state and thus the resulting FEF depend on the acceptor strength of substituent R in the 5-position, reflecting the ET nature of the quenching process. de Silva *et al*. also designed a series of triaryl-Δ^2-pyrazoline-type fluorescent probes with different selectivities for physiologically important alkaline-earth metal ions such as Ca^{II} and Mg^{II}.[17] For example, introduction of Tsien's Ca^{II} receptor BAPTA yielded **8a** and its bichromophoric analogue **8b** which both display some of the most dramatic FEF induced by



7: X = H or OCH_3,

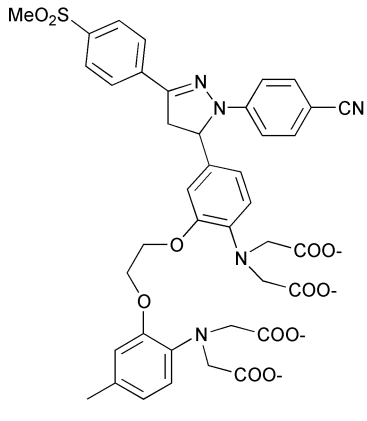
Y = H, OCH_3, phenyl, or CN,

R = 2-, 3-, 4-pyridyl or 2-, 4-COOH-phenyl

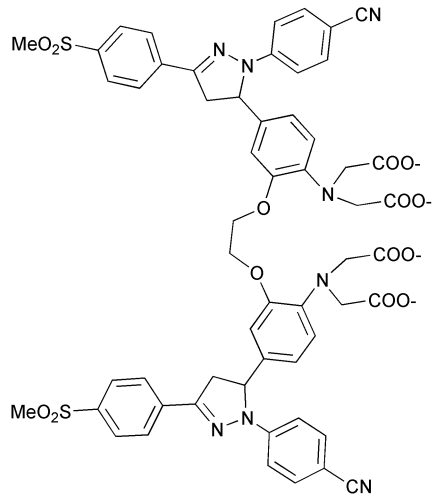
7a: X = H, Y = CN, R = 3-pyridyl

biologically relevant levels of Ca^{II} with FEF = 92 and 180 in water for **8a**–Ca^{II} and **8b**–Ca^{II}.[17] The favorable signal changes are most likely due to the strongly diminished fluorescence in the 'OFF' state. Additionally, both compounds show a good selectivity for physiological levels of Ca^{II} against those of Mg^{II} and protons. Their only spectroscopic drawbacks are the comparatively low fluorescence quantum yields in the 'ON' state ($\Phi_f = 0.100$ and 0.045 for **8a**–Ca^{II} and **8b**–Ca^{II}) and, to a minor degree, the spectral positions of their emission bands in a region where the red edge of auto-fluorescence from a biological background can still be encountered. Switching 'ON' of the fluorescence of **8a** and **8b** by a significant inhibition of the ET process occurs *via* a Ca^{II}-induced conformational change of the acyclic receptor. The entry of the cation decouples the iminodiacetate moieties from the (bis)alkoxyphenyl backbone, entailing a large increase in the receptor's oxidation potential. The lower Ca^{II} binding constant of bichromophoric **8b** is due to inter-fluorophore steric effects. Introduction of the receptor bis(2′-anisyl) diaza 15-crown-5 led to compounds **9a** and **9b** which show a moderate fluorescence enhancement of 9 and 16.3 with Na^{I} in MeOH–water, while discriminating against K^{I} with only Li^{I} interfering.[18]

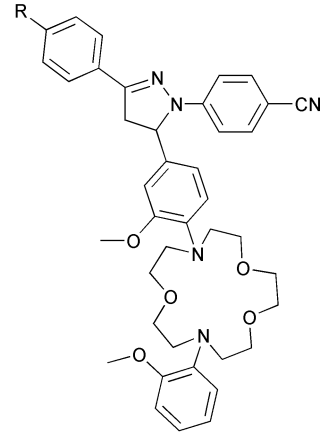
With the incorporation of the more strongly electron accepting heterocyclic 3-benzothiazol-2-yl (BT)[14] and 3-(*N*-phenyl-1,8-naphthalimid-4-yl) (Ph-NI) fragments,[19] the absorption and emission bands of the Δ^2-pyrazoline chromophore can be shifted into the instrumentally and analytically advantageous vis–NIR region. Employing monoaza crown ethers as 5-substituents, this concept has been successfully utilized to design vis–NIR fluorosensors for either 'hard' alkaline-earth and alkali metal ions (**10a** and **11a**) or 'soft' heavy and transition metal ions (**10b**, **11b**). Following the 'OFF'–'ON' switching in a similar fashion as shown for de Silva's cation probes, an ET



8a

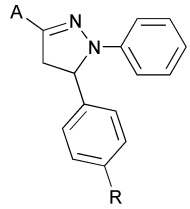


8b

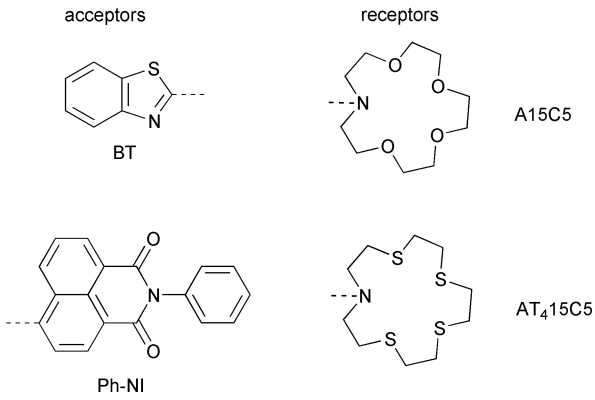


9a: R = CN

9b: R = SO_2Me



10a: A = BT, R = A15C5
10b: A = BT, R = $AT_4$15C5
10c: A = BT, R = H
11a: A = Ph-NI, R = A15C5
11b: A = Ph-NI, R = $AT_4$15C5
11c: A = Ph-NI, R = H



from the electron-rich 5-anilino substituent renders the free systems only weakly fluorescent. Upon cation coordination, even in the case of known fluorescence quenchers such as Pb^{II} and Hg^{II}, the ET is suppressed and a typical increase in fluorescence quantum yield and lifetime is found. For example, FEF of 44 and 43 as well as 34 and 47 are obtained for the Ca^{II} as well as Pb^{II} complexes of **10a** and **11a**, respectively, and the thia crown analogues **10b** and **11b** show FEF of 6.5 or 2.2 and 15 or 20 upon binding to thiophilic Ag^{I} or Hg^{II} in acetonitrile. These effects clearly demonstrate the importance and future analytical potential of fluoroionophore structures that are constructed according to the key guidelines emphasized in this article. Furthermore, as follows from a comparison of the fluorescence quantum yields of **10a** (Φ_f = 0.016), **10b** (Φ_f = 0.10), **11a** (Φ_f = 0.003) and **11b** (Φ_f = 0.007) with those of the unquenched parent compounds (**10c**: Φ_f = 0.72; **11c**: Φ_f = 0.18), the exploitation of 'sensitive' ET quenching reactions requires an exact knowledge of the actual donor and acceptor redox potentials.[14] A comparison of the two parent compounds, however, reveals also a limiting factor of 1,3,5-triaryl-Δ^2-pyrazoline-type systems (and other CT fluorophores). When aiming at an utmost switching efficiency as well as a shift of the regions of absorption and emission into the instrumentally and analytically desirable far-visible–NIR, lower overall fluorescence quantum yields in the 'ON' state have to be accepted (**11c** *cf.* **10c**). This intrinsic counter-effect is based on the reduced energy gap between the excited and the ground state, yielding diminished fluorescence quantum yields with increasingly red-shifted emission maxima according to the energy gap rule.[14]

So far, we have only discussed 5-receptor-functionalized 1,3,5-triaryl-Δ^2-pyrazolines but, as can be deduced from the synthetic scheme (Scheme 4), in principle, also the 1- and 3-positions can be equipped with analyte-responsive units. However, up to now, only very few examples of such systems have been reported in the literature. Rivett *et al.* found a dramatic decrease in fluorescence quantum yield in apolar as well as polar solvents upon introducing donor groups to the 1-*p*-position (Φ_f = 0.36 *vs.* 0.91 for 1-aminophenyl-3-cyanophenyl-5-phenyl-Δ^2-pyrazoline *vs.* 1,5-diphenyl-3-cyanophenyl-Δ^2-pyrazoline), the mechanistic details of the quenching process not being commented on.[21] Since, to the best of our knowledge, no examples of 1-*p*-receptor phenyl-substituted fluorescent Δ^2-pyrazoline probes have yet been reported, the potential of this group of compounds remains to be investigated. In contrast, a potentially attractive extension of the presented design concept to bifunctional probes and 'logic gates' follows from the results reported in refs. 14 and 22. As the consecutive complexation behavior of **10b** with Hg^{II} suggests, 3-receptor-substituted compounds could be powerful molecular reporters. In this case, complexation at the 5-receptor at low metal ion concentrations is followed by the formation of a 3-acceptor chelate at higher metal-to-ligand ratios (Fig. 1).[14] As was shown by us in mechanistic studies employing a series of model compounds, the second ligating site consists of the nitrogen atom of the BT moiety and N(2) of the pyrazoline chromophore. The result of this coordination-enhanced acceptor strength is the appearance of a second red-shifted absorption and emission band. Thus, with the proper choice of 3- and 5-substituents, even bifunctional Δ^2-pyrazoline fluorosensors forming two types of spectroscopically distinguishable complexes can be designed. Such features enable either an extension of the dynamic working range for metal ion sensing or an independent recognition but cooperative report of cations with different binding preferences. A very similar strategy was employed when de Silva *et al.* utilized 1,3-dipyrid-2-yl-5-phenyl-Δ^2-

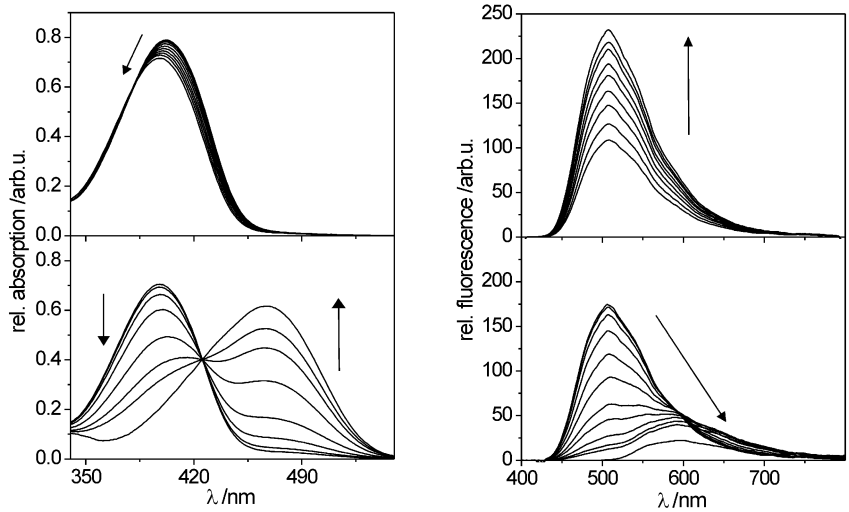


Fig. 1 UV–vis spectrophotometric (left) and fluorometric (right) titration spectra of **10b** with Hg^{II} in acetonitrile. Hg^{II} : **10b** ratios between 0.02–1 (upper parts; excitation at 400 nm) and 2–100 (lower parts; excitation at 425 nm). The emission spectrum of lowest intensity in the lower right graph corresponds to the spectrum of Hg^{II}–**10b**–Hg^{II}, excited at 480 nm (adapted from ref. 14).

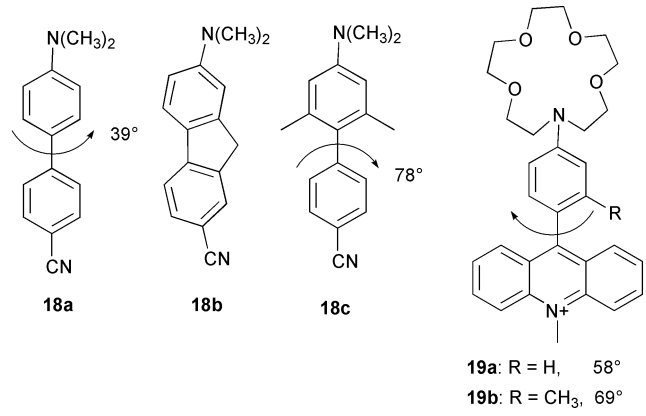
pyrazoline as one of the input junctions of a logic 'NOR' gate.[22]

5 Preoriented and electronically decoupled molecular subunits

A 'switching' of the fluorescence output can be also achieved in 'zero' spacer donor–acceptor-functionalized dyes which usually belong to the class of asymmetric biaryls. Since the chemical nature of biaryls is already characterized by a restricted flexibility—only the central σ-bond connecting both subunits is free to rotate—any further steps to rigidize the supramolecular structure are not essentially necessary for improved probe design. Incorporating the cation-responsive receptor into one of the two fragments, the conceptual prerequisite requires a minimization of the electronic interaction of the subunits by arranging both moieties in a (more or less) orthogonal position. Although the efficiency of the indication process, in principle, is expected to be a function of the interannular twist angle, the fluorescent sensors and switches belonging to this type of probes can be divided into two categories with respect to fluorescence signal expression: fluorescent reporters populating two emissive or only one emissive and a non-emissive excited state.[23] For a better understanding, the interannular twist angles as obtained for the AM1-optimized ground state geometries of all the compounds discussed in this section are included for the respective chemical structures (AM1, Ampac V6.55, Semichem, Inc.).

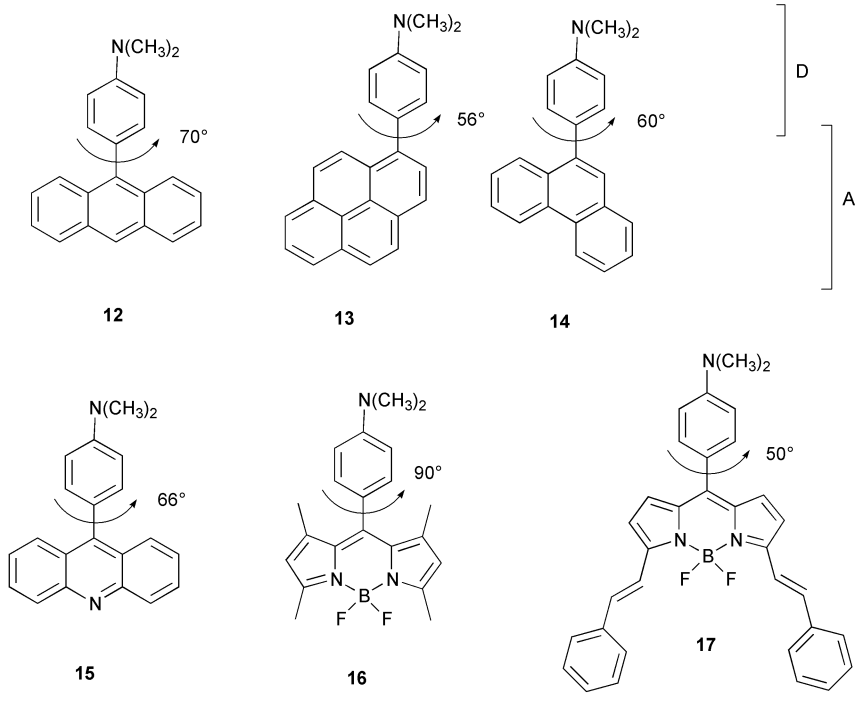
In most of the dyes described to date, the analyte-responsive receptor is an anilino derivative, representing the donor moiety of these D–A biaryls. Much less frequently, phenol- or naphthol-substituted biaryls were employed.[24] Since the indication mechanism of anilino- and phenol-appended biaryls is very similar, the nitrogen containing compounds will be exemplarily introduced here. In principle, when equipped with the simple dimethylamino group, all of these compounds—dyes **12**–**17** serving as representative examples—could be used for proton sensing. As the detailed mechanistic background on excited-state charge or electron transfer in such compounds has been recently reviewed,[23] we will refer only to those molecules that have been actually employed in sensing and switching applications and will restrict the present discussion to mechanistic features important for the observed signaling processes.

Due to the specific architecture of asymmetrically substituted biaryls, the optical transitions can either be localized on the respective fragments or can be of charge-transfer character, involving the whole D–A chromophore. For the majority of the dyes with a polyaromatic or heterocyclic chromophore, the localized transitions lowest in energy are those centered on these electron-deficient fragments, commonly at $\bar{\nu} < 31000$ cm^{-1}.[23] The aniline-localized transitions are much higher-lying in energy (typically at 37000–38000 cm^{-1}) and their influence can be neglected in the present considerations. (The same accounts for acceptor-localized transitions which are polarized perpendicularly to the D–A molecular axis.[23]) Only for the substituted biphenyls **18a**–**c** the situation is slightly different,



since the respective transitions in the parent compounds or fragments biphenyl, cyanobiphenyl, and dimethylaminobiphenyl or the respective fluorenes are found in the same energy region of 33000–40000 cm^{-1}.[25] Nonetheless, **18a**–**c** are excellent examples for illustrating the influence of preorientation and electronic decoupling on the sensing features of D–A molecular reporters with a 'zero' or 'virtual' spacer.

The fluorene derivative **18b** is fixed in a planar conformation and, after charge-transfer excitation, the molecule shows a strong charge-recombination (CR) fluorescence. Upon protonation of the anilino group, the absorption spectrum changes



drastically. The CT band disappears almost completely and a mixture of ^1L_a- and ^1L_b-type cyanofluorene-localized bands is found. Apparently, the proton removes the anilino nitrogen's lone electron pair from the π-system. In mixed aqueous solvents, the absorption spectroscopic changes are concomitantly followed by the disappearance of the CT emission at the expense of a new, energetically higher-lying and localized (usually termed 'locally excited' or 'LE') fluorescence band. Consequently, these features allow the employment of **18b** for absorption AND emission ratiometric pH-sensing in a comparatively large dynamic working range between pH 0–4. A ratiometric signaling behavior is advantageous as such systems are internally calibrated, *i.e.*, fluctuations of the light source and photobleaching do not affect the signal ratio of bound and unbound dye and thus do not lead to erroneous intensity changes. The high molar absorptivities as well as fluorescence quantum yields of all the species involved render **18b** a very sensitive fluorescent pH probe. The D–A derivative of biphenyl, **18a**, shows a very similar behavior since, after CT excitation, the molecule undergoes an ultrafast reaction from a *ca.* 40° pretwisted initially excited to a planar charge-separated state. In analogy to **18b**, population of the latter is followed by CR fluorescence. Again, the presence of protons also entails a dual absorption–dual emission behavior as observed for **18b**. In contrast, initially excited **18c**, which is internally pretwisted for *ca.* 80°, funnels towards a highly twisted CT* species with a reactive biradicaloid electronic structure. Thus, already in protic non-aqueous solvents such as alcohols, the CT fluorescence is strongly diminished. Upon addition of acid, the $D^{+\cdot}$–$A^{-\cdot}$ species strongly attracts protons from the environment and efficiently forms a D^+–A–H species. For this species, deactivation by the CR fluorescence channel is unaccessable and [$D^{+\cdot}$–$A^{\cdot}$–H]* decays *via* a non-radiative charge shift to the ground state (D–$A^+\cdots$H). Thus, only LE emission is observed. With the examples of **18a–c** we could clearly demonstrate, how careful preorientation of the molecular subunits allows signaling systems with specific properties to be obtained. Whereas **18a**,**b** show a favorable ratiometric CT 'ON'–'OFF' and LE 'OFF'–'ON' switching behavior in mixed aqueous solvents, highly preoriented **18c** acts as a very efficient LE 'OFF'–'ON' probe already in non-aqueous protic solvents.

The acridinium-substituted fluorosensors for metal ions **19a**,**b** represent the second group of D–A biarylic compounds, molecules with only a single potentially emissive excited state. In the absence of an analyte, **19a**,**b** show absorption spectra with contributions from the acridinium-localized as well as the CT band (Fig. 2).[16] The intensity ratio of these transitions correlates

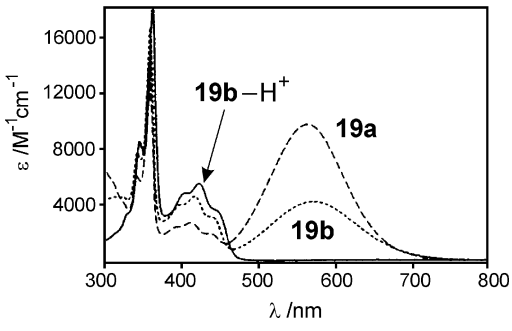
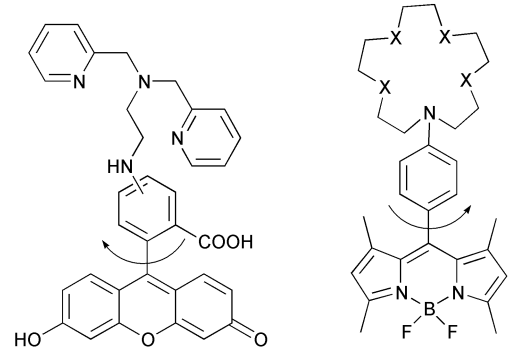


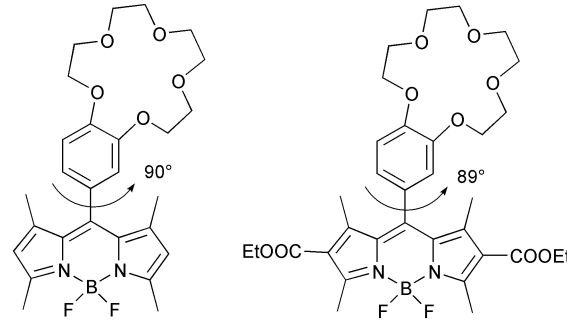
Fig. 2 Normalized absorption spectra of **19a**, **19b**, and **19b**–H^+ in acetonitrile at 293 K (adapted from ref. 16).

with the degree of preorientation, *i.e.*, the twist angle between the molecular subunits, and less twisted **19a** shows a much stronger intramolecular charge transfer component. Excitation in both absorption bands does not result in fluorescence. This absence of CT as well as acridinium-localized LE emission, the latter centered at *ca.* 510 nm, has been ascribed to the ultrafast population of a non-emissive (twisted intramolecular) charge-transfer (TI)CT state.[16] Complexation or protonation leads to the abstraction of the nitrogen atom's lone electron pair and strongly diminishes the CT absorption band (Fig. 2). For metal ions, the magnitude of this effect depends on the nitrogen-coordinating ability of the cation and reflects donor atom preferences of the target as well as geometrical or conformational host–guest fitting parameters. As a consequence of this 'switching off' of the intramolecular CT process, the acridinium fluorescence is revived. Although spectrally very similar, the importance of preorienting the subunits to achieve a high fluorescence output follows from a comparison of the photophysical properties of the Ag^I complexes of these probes, Φ_f = 0.014 and 0.072 for **19a**–Ag^I and **19b**–Ag^I in acetonitrile, respectively. Moreover, the difference in interannular twist angle of θ = 69° for **19b** as compared to 58° for **19a** does not only increase the switching efficiency but entails a better preorganization of the receptor for complexation, which is manifested by the higher complex stability constant log K_S = 3.46 for **19b**–Ca^{II} as compared to log K_S = 3.06 for **19a**–Ca^{II}.[16]



20a: 5-substituted, 87°
20b: 6-substituted, 83°

21a: X = O, 89°
21b: X = S, 90°



21c **22**

The Zn^{II}-selective 'zero' spacer probes **20a**,**b**[26] as well as the boron–dipyrromethene (BDP)-based molecular proton- and Na^I-/K^I-responsive reporters **17**[27] and **22**[28] principally operate in a very similar way. In the unbound state, no CT and only a very weak LE emission are found, the latter being characteristic for the fluorescein or substituted BDP fluorophores, respectively. In the presence of the respective analytes, the LE fluorescence of the indicators is switched 'ON', resulting in high fluorescence enhancement factors of, *e.g.*, 17 for **20a**–Zn^{II}, 51 for **20b**–Zn^{II}, 37 for **22**–Na^I, or *ca.* 2000 for **17**–H^+.[26–28] For the compound lacking neighboring methyl groups, the difference in absorption between **17** as compared to **19a** originates from intrinsic chromophoric properties. In contrast to **19a**, where the maxima of localized and CT transitions are found at 420 and 560 nm in acetonitrile,[16] for **17**, the position of the acceptor-localized transitions of the more extended heteroaromatic BDP-type moiety is shifted to lower energies and the

situation is even reversed: in acetonitrile, for instance, the CT transition centered at 545 nm occurs at higher energies as the fluorophore-localized absorption at 620 nm.[27]

Recently, we were able to demonstrate that good switching efficiencies can even be obtained with considerably weak donor–receptor groups.[28] Whereas anilino or phenolate groups have a strong tendency to transfer or shift charge to an acceptor moiety in the S_1 state, alkoxyphenyl or benzo crown ether substituents are often only weakly or even not at all CT- and ET-active in combination with conventional acceptor groups. For example, **21c** already displays a strong fluorescence in the unbound state and is thus a very poor candidate for obtaining analyte-enhanced fluorescence signals. However, to gain sensitivity for alkali metal ions with respect to aminophilic heavy and transition metal ions, the exchange of, for instance, aza oxa crowns for benzo crowns is highly desirable. Instead of searching for new and more powerful acceptor moieties, we opted for the elaboration of an already reliable dye architecture and introduced electron accepting ester groups to the BDP moiety, yielding the benzo crown-functionalized switch **22**. As revealed by a less negative reduction potential $E_{1/2} = -1250$ mV for **22** as compared to $E_{1/2} = -1575$ mV for **21c**, the acceptor unit of **22** is now strong enough to trigger an ET-like quenching process, reminiscent of those in **21a**,**b**.[28] Alkali metal ion-binding to **22** is then accompanied by an enhancement of the BDP-localized emission, *e.g.*, 37-fold for **22**–Na^{I} in methanol.

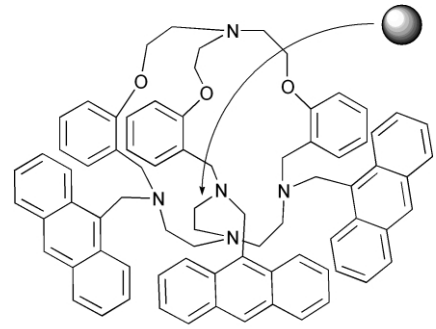
Although being water-soluble, a disadvantage of the sensing features of **20a**,**b** is their proneness towards fluorescence quenching when binding to transition metal ions such as Cu^{II}.[26] Apparently, when compared to closely related **21b**, which undergoes a 2500-fold fluorescence enhancement upon binding to Cu^{II} in acetonitrile, the high preorientation of **20a**,**b** seems not to be sufficient to prevent direct electronic interaction between the quenching metal ion–receptor complex and the fluorophore *via* a spacer topology with four flexible σ-bonds, Ph–NH–CH_2–CH_2–NR_2. By contrast, in **21b**–Cu^{II}, the potentially quenching ion is tightly held by the rigid –C_6H_4–N< spacer at a rather long distance from the fluorophore, obviously rendering such an electron transfer interaction during the lifetime of the excited state unfavorable.

Having already introduced model compounds of the group of biaryl-type fluorescent sensors and switches that populate two potentially emissive excited-state species of LE and CT character, we complete this section with D–A-substituted, highly preoriented molecular reporters **21a**,**b**.[13,29] In analogy to **22**, for these dyes the orbital overlap of donor and acceptor is negligible and the absorption spectrum of the molecules is a linear combination of the composite spectra of their subunits. However, in contrast to **22** as well as **17**, **21a**,**b** and also **16** show well-separated dual emission in many weakly to highly polar solvents.[13,29] Whereas the narrow and structured fluorescence band at *ca.* 510 nm does not change its shape or position and can thus be attributed to the BDP-localized emission, the second emission band is broad and unstructured. Additionally, it is strongly shifted to the red and increases in size, *i.e.*, in full width at half maximum, with increasing solvent polarity. Time-resolved fluorescence experiments and global data analysis revealed that this red-shifted transition can be assigned to the radiative decay of a highly twisted ^{1}CT species.[13] Furthermore, the position as well as the relative contribution of the CT emission to the overall fluorescence intensity was found to correlate with the donor strength of the basic substituent, the CT process increasing in the order **21b** < **21a** < **16**. The sensing features of these highly decoupled dyes are outstanding. Whether aiming at problematic analytes such as Na^{I}, commonly known to yield only weak complexation-induced effects, or strongly quenching heavy metal ions such as Hg^{II} as well as paramagnetic transition metal ions such as Cu^{II}, analytically advantageous fluorescence enhancement factors of 485 (**21a**–Na^{I}),[13] 5900 (**21b**–Hg^{II})[29] or 2500 (**21b**–Cu^{II})[29] can be obtained in acetonitrile.

6 Receptor-based conformational constraints and guest-mediated reconfiguration

After the presentation of two general concepts that predominantly profit from the careful design of dye architecture and tuning of the photophysical signal generation and transduction processes, we now introduce some striking examples for the closely related yet mechanistically different strategies of *receptor-based* or *analyte-induced* rigidization or preorientation in fluorescent molecular devices.

A first promising approach to achieve dramatic chelation-enhanced fluorescence not only with diamagnetic metal ions, but also with commonly quenching heavy and paramagnetic metal ions and rationally designed ET probes was realized by Bharadwaj *et al.*[30] These researchers improved the 'classical' ET-active molecular format of compounds such as **3** by using a cage-like cryptand receptor framework. The resulting molecular device (*e.g.*, **23**) has a highly shielding cavity for the potentially



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quenching ion to be bound and the ligand's donor atoms are three-dimensionally prearranged to guarantee optimum binding for tetracoordinating potential quenchers such as Cu^{II} or Co^{II}.[30] Besides a possibly more covalent character of the metal ion–nitrogen atom bond, the advantageous, up to a several hundred-fold fluorescence enhancement in the presence of various paramagnetic transition metal ions was attributed to the reduced redox activity of the incorporated ion. According to Bharadwaj *et al.*, the preorientation of the coordinating donor atoms of the enveloping receptor prevents geometric rearrangements which are essential for stabilizing the different coordination spheres of other oxidation states of the respective paramagnetic cation in the course of an ET process. Under these premises, *i.e.*, with a receptor-shielded quencher and minimized fluorophore–cation interaction, the nature of the spacer loses its importance and high analyte-induced emission signals can be also obtained with this otherwise conventionally designed ET probe containing a methylene spacer and an anthracene fluorophore.

A different attempt to increase selectivity and sensitivity by invoking a switch-like change of the fluorescence intensity has been realized in systems relying on guest-mediated intramolecular reconfiguration. The supramolecular furniture of these fluorescent sensor molecules is rather diverse. Accordingly, the signaling mechanisms are also diverse and the systems we choose to present here are reminiscent of advanced successors of conventional exciplex/excimer forming probes (**24**–**26**), invoke specific cation–fluorophore interaction (**27** and **28**) or are related to dual emissive biaryl-type molecules (**29**).

The preorganization of the anthracene units is the key step for the fluorescence changes in **24**, but contrary to most excimer forming sensor systems where photocycloadditions are unwelcomed, this otherwise interfering photoreaction is the

indication of choice here.[31] Moreover, since **24** was conceived as a dual analyte signaling system and, in particular, as an 'OR' logic gate, Tucker *et al*. introduced a ditopic ligating site in such a way that coordinative engagement of each of the two different targets strongly enhances the reaction rate. The photochemical products are deprived of the extended π-conjugation of the anthracene ring systems and thus, **24** behaves as a fluorescence 'ON'–'OFF' switch. A peculiarity of **24** is the analyte-responsive behavior indicated by the thermal dissociation of this reversible reaction cycle. Of both cations, only Na^{I} that actually resides in close vicinity of the reaction center slows down the dissociation significantly. Remotely bound Hg^{II} induces only minor kinetic changes. By reporting on the closely related *meta* substituted bipyridyl derivative, these researchers demonstrated the importance of the sterical aspect for such probes. As compared to the photoproduct of the *ortho* derivative **24**, the *meta* substituted photoproduct suffers from enhanced strain in the polyether units and thus the reaction rates are unaffected by the presence of both cations, hampering sensory applications of the latter compound.

The guest-mediated reconfiguration of **25** is of a completely different nature yet the resulting quenching of the free probe's excimer fluorescence is outstanding (Fig. 3).[15] Upon binding to

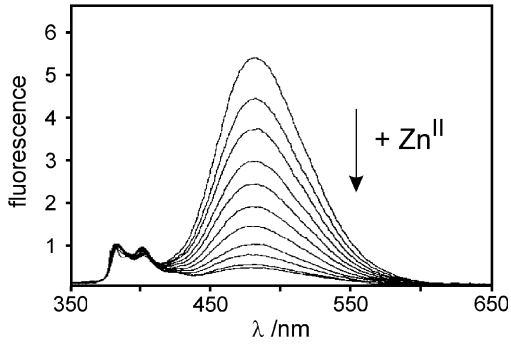
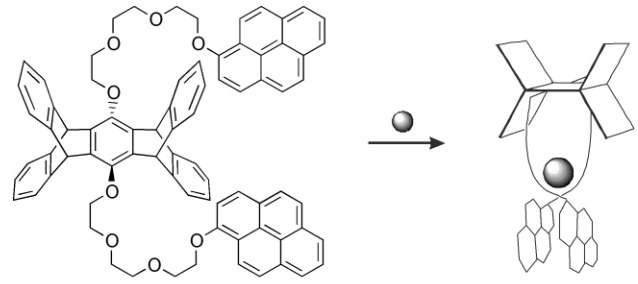


Fig. 3 Fluorescence titration of **25** with Zn^{II} in an acetonitrile–chloroform (1 : 1) mixture at 298 K (adapted from ref. 15).

Zn^{II}, the conformationally sophisticated receptor–spacer unit invokes a triple ring flip of the perhydroanthracene moiety. This conformational rearrangement separates the two pyrene units, drastically increasing the monomer-to-excimer emission band ratio.[15]

The opposite phenomenon is encountered when adding Cu^{II} ions to an acetonitrile solution of **26**, *i.e*., coordination of the



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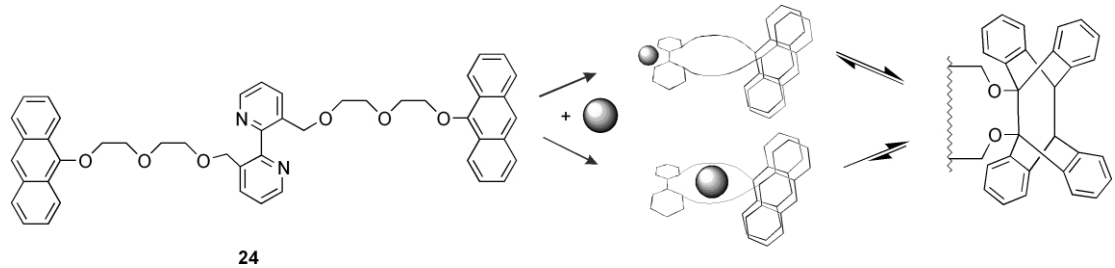
metal ion greatly enhances pyrene excimer emisson.[32] A particular feature of the visualization of Cu^{II} with **26** is the blue shift of the typical excimer band. The strong **26**–Cu^{II} complex brings the pyrene moieties already together in the ground state, but the dimensions and structure of the spacers prohibit a total overlap and optimum alignment of the two chromophores. Thus, after excitation, relaxation of the initially formed and only partially overlapping pyrene excimer to an energetically stabilized excimer with optimum overlap is prevented and the binding event is accompanied by a broad emission band at 440 nm instead of 475 nm, commonly found for optimum aligned pyrene excimers.[32]

The 'ON'–'OFF' signaling processes of **27**[33] and **28**[34] are very similar, only their selectivity is reversed. Whereas **27** predominantly recognizes Hg^{II} and Cu^{II} causes weaker effects, **28** prefers Cu^{II} over Hg^{II} while other potentially interfering transition metal ions such as Ni^{II}, Co^{II}, Fe^{II} or Zn^{II} lead to little or no change. The principle of operation is based on enhanced cation–fluorophore interaction. Accommodation of the respective metal ions by the binding unit strongly diminishes the emission of the fluorophore, resulting in an 'ON'–'OFF' switching effect. The differences as compared to other systems introduced here is the flexible composition of the sensor molecules. Especially in **28**, no preoriented or constrained spacer and receptor units discriminate between the different analytes but simply the metal ion-dictated formation of the cation–receptor chelate decides on the amount of the resulting quenching interaction. As for **28**, quenching was found to proceed *via* an energy transfer mechanism, the intrinsic electronic nature of the metal ion additionally plays a role. In



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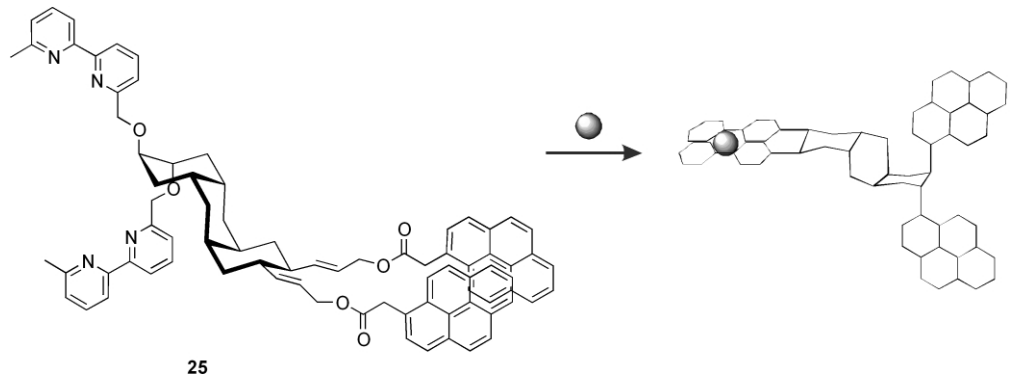

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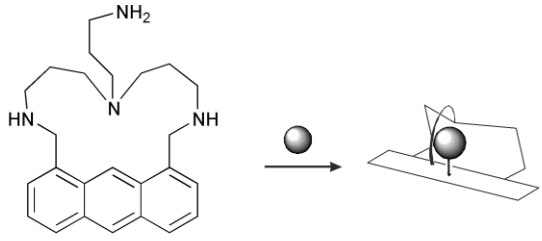

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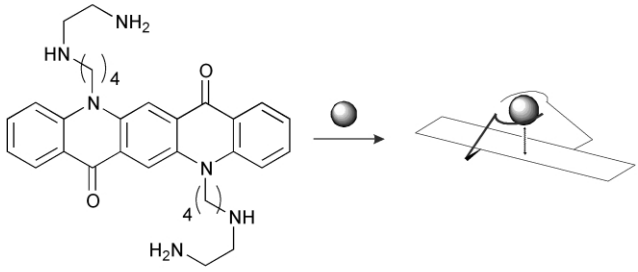

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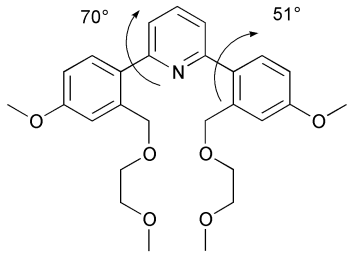
this respect, Klein *et al*. attributed the stronger effect of Cu^{II} as compared to the d^{10} metal ion Hg^{II} mainly to its paramagnetic properties.

The model compound which will complement the review, **29**, is an example of guest-mediated conformational restriction of a



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biaryl-type sensor molecule, resulting in modulations of chromophore-localized and charge-transfer transitions.[35] In this respect, the signal expression is reminiscent of the behavior described in Section 5 for **18a**,**b**. In the unbound state, only LE fluorescence at *ca*. 350 nm is found for **29** in acetonitrile. (Note that in contrast to **18a–c** and with respect to the features discussed in connection with **21c** and **22**, no anilino donor groups are present in **29**.) Depending on the ability of the cationic guest to keep the arylic subunits of the host in a fixed position, various ion-induced changes can occur. For example, whereas Li^{I} strongly enhances the LE emission, binding of Ca^{II} additionally leads to an intense CT band centered at *ca*. 450 nm. Coordination of Mg^{II} on the other hand reduces the LE emission but is accompanied by a pronounced appearance of a CT emission band. Since the positively charged metal ions also strongly affect donor and acceptor properties, especially increasing the latter, not only conformational restriction but CT induction contributes to the advantages of **29** for fluorometric main group metal ion discrimination in a ratiometric, dual emission mode.

7 Future prospects

The fluorescent molecular sensors and switches introduced here suggest that the key concepts of rigidizing, preorienting, and electronically decoupling the functional subunits of fluorophore–spacer–receptor systems are, in general, promising strategies for future rational design of efficiently signaling molecular reporters. This also includes the predictability of the molecule's properties from the spectroscopic, electrochemical, and analyte recognition properties of the parent modules. For instance, the 'box-of-bricks' construction principle of the 1,3,5-triaryl-Δ^2-pyrazolines readily allows tailor-made receptors to be combined with instrumentally and analytically advantageous fluorophores. Equipping the 1,3-fluorophore of **11** with the Ca^{II}-selective 5-receptor of **8** should, for instance, yield specific fluorosensors communicating in the red–NIR. As already mentioned above, future research on this particular class of compounds should also spread out beyond the conventional 5-receptor-functionalized probes. Besides a step towards bi- or multi-analyte indication, heading into such a direction offers the opportunity to elucidate the potential of these dyes for combined ET/CT signaling and may introduce new powerful ratiometric sensing modes. The future potential of the highly decoupled biaryl-type reporters with their partly extraordinary switching properties predominantly lies in two areas, (i) controlled functionalization for applications in specific biological media (*e.g*., signaling the directed membrane transport of certain analytes) and (ii) advanced receptor design to exploit their favorable features for the monitoring of other, more weakly interacting guests such as anions or neutral molecules commonly failing to switch an output signal. Concerning chromophore chemistry, the use of electron-deficient fluorophores to invoke fast quenching processes as demonstrated in **16** or **21a**,**b** is highly recommended and stresses the fact that, besides steric control, special attention should be devoted to donor and acceptor properties. Although less predictable, researchers aiming at unperturbed 'OFF'–'ON' switches should keep in mind indicator molecules such as **17** or **19b** that rely on an interplay of an essentially non-emissive and a highly emissive excited state. In the case of excimer/exciplex forming probes, especially sensor molecules with a remote binding site (*e.g*., **24**, **25**) seem to be able to generate strong analyte-promoted fluorescence in the presence of very different chemical species including potential quenchers.

On a more general scale, there are a few requirements or desirable properties which the chemist as a designer should be alert to in future probe research. (i) Absorption and emission, preferably with a large Stokes shift, should at best lie well within the vis–NIR spectral region. (ii) 'Internally calibrated' dual fluorescence (and dual absorption) signal expression facilitates user-friendly and 'robust' ratiometric sensing. (iii) Water solubility is important for leaving the mechanistic and entering the real (*in situ* analytical) world. (iv) Incorporation of fluorescent probes into rigid matrices, either by embedding or by covalently attaching the sensor molecules, is a major applicational task and in this case, one has to be aware of the critical role that the micro-environment of the network plays for many photophysical and photochemical processes relying on certain intramolecular motions.

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